

associated with activation of brain regions mediating emotion and fear as evidenced by upregulation of the pre-frontal limbic regions in the hand OA group. Development of future therapies could be targeted at modulating activation of central pain processing centres in OA to improve pain management in patients.

81 RACIAL DIFFERENCES IN PAIN REPORTING IN SUBJECTS WITH OR AT RISK FOR SYMPTOMATIC RADIOGRAPHIC KNEE OSTEOARTHRITIS: DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: African Americans have a higher prevalence of both radiographic and symptomatic radiographic knee OA (KOA) than Caucasian Americans. While the reasons for this disparity are poorly understood, differences in risk factor patterns, coping mechanisms, and/or pathoanatomic characteristics have been suggested. Less is known, however, about differences in clinical examination features of the knee between the two groups and whether such differences may explain the greater frequency of pain and higher pain reporting in African Americans with knee OA.

Methods: Racial differences in pain reporting and severity as well as findings on physical examination of the knees were assessed using baseline data from subjects enrolled in the Osteoarthritis Initiative (OAI). The OAI is a multicenter, longitudinal observational study initiated in 2004 to examine biomarkers and risk factors for clinically significant knee OA. This analysis included 372 African Americans and 974 Caucasians with symptomatic radiographic KOA in at least one knee (Progression subcohort) and 495 African Americans and 2703 Caucasians at risk for developing symptomatic radiographic KOA (Incidence subcohort). In the current analysis, 3 distinct measures of pain severity (KOOS [0–100], WOMAC [0–20] and NRS [0–10]) and 7 physical exam findings were analyzed for each knee initially using bivariate methods and, subsequently, using multiple variable linear and logistic regression models, respectively, adjusting for body mass index, age, gender, income, depression score, and education level.

Results: African Americans reported higher pain severity than Caucasians in both knees for each assessment measure analyzed (Both

subcohorts: $P < 0.0001$ for all comparisons) (Table 1). Furthermore, there were significant differences in physical examination findings between African Americans and Caucasians for the majority of physical examination features in both knees in both subcohorts (Table 2). After adjustment for these differences in physical examination findings as well as multiple confounding variables (see above), African-Americans continued to report greater pain severity than Caucasians both in the absence of symptomatic radiographic KOA (Incidence subcohort: $P < 0.0001$ for all pain outcomes) and in the presence of symptomatic radiographic KOA (Progression subcohort: $P < 0.05$ for all pain outcomes except left knee pain in the last 7 days measured by NRS).

Conclusions: These results demonstrate that African Americans report more pain than Caucasians, even after adjusting for sociodemographic factors, depressive symptoms and obesity, and that, regardless of the cause, this difference cannot be explained by findings on physical examination. Further analyses should examine the potential role of coping mechanisms, findings on magnetic resonance imaging of the knees and access to both nonpharmacologic and pharmacologic interventions.

82 P-188, ANTI-TNF- α AND OP-1: A NOVEL TRIAD IN THE PREVENTION OF POST-TRAUMATIC OSTEOARTHRITIS

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Purpose: This continuing study investigated the utility of individual or combined pro-anabolic and anti-catabolic biologic interventions targeting chondroprotection and cartilage repair in an *ex vivo* acute injury model of post-traumatic osteoarthritis (PTOA) in human ankle cartilage.

Methods: Fifteen normal tali obtained from organ donors through the Gift of Hope Organ and Tissue Donor Network were impacted using a 4mm cylindrical indenter with 600N following a model previously described. 8mm cartilage explants comprising the 4mm impacted core and the immediately adjacent 4mm non-impacted ring were cultured for 14 days in the presence of 5% serum and osteogenic protein-1 (OP-1), two doses of interleukin (IL)-1 receptor antagonist (IL-1RA); tumor necrosis factor- α (TNF- α) antagonist, N-Acetyl-L-Cysteine (NAC), two pan caspase inhibitors, P-188 surfactant, or the combination of P-188, OP-1, and anti-TNF- α (Triad). Controls included untreated impacted and non-impacted explants. Tissue and media samples were collected on days 0, 2, 7, and 14 to assess cell survival and metabolic activity. Samples were analyzed for cell viability (live/dead assay), apoptosis (Tunel assay), matrix integrity (Safranin O staining, Mankin score), and proteoglycan synthesis and content.

Results: A single impact to human articular cartilage resulted in cell death/apoptosis within the impacted core, which if untreated, expanded to the adjacent non-impacted ring. Sustainable significant increase in cell survival was observed under the treatment with OP-1 and more so in Triad (24% and 43%, $p < .001$ respectively). These same treatments were the most significant in stimulating PG synthesis (5-fold and 6-fold, $p < .001$ respectively). Two of the triad components (TNF- α antagonist and P-188), when given alone did not show any significant effect on PGs. P-188, OP-1, and TNF- α antagonist in combination showed a better preservation of matrix integrity as evidenced by normal pattern of Safranin O staining throughout the entire section. Mankin score was within range of non-impacted control and was about 2-fold lower than the impacted

Table 1
Knee pain by race/ethnicity for subjects in the Incidence and Progression cohorts

Cohort	Incidence	Incidence	Progression	Progression
Race/ethnicity	African American	Caucasian	African American	Caucasian
Right knee pain, NRS	3.06 (2.67)	1.85 (2.16)	4.76 (4.76)	3.49 (2.59)
Right knee pain, WOMAC	3.41 (3.71)	1.50 (2.25)	6.03 (4.37)	3.69 (3.36)
Right knee pain, KOOS	80.1 (19.0)	89.5 (12.5)	65.4 (21.5)	76.1 (17.7)
Left knee pain, NRS	2.96 (2.88)	1.74 (2.19)	4.60 (3.08)	3.49 (2.76)
Left knee pain, WOMAC	3.34 (4.08)	1.36 (2.35)	5.80 (4.89)	3.46 (3.62)
Left knee pain, KOOS	80.8 (20.7)	90.6 (13.0)	67.4 (24.2)	77.7 (19.1)

Table 2
Knee exam findings by race/ethnicity for subjects in the Incidence and Progression cohorts

Cohort	Incidence	Incidence	Progression	Progression
Race/ethnicity	African American	Caucasian	African American	Caucasian
Right patellar tendon pain	106 (21.5%)	283 (10.6%)	104 (28.8%)	174 (18.0%)
Right knee flexion pain	83 (16.8%)	285 (10.8%)	141 (39.2%)	279 (29.0%)
Right lateral jointline tender	125 (25.3%)	459 (17.2%)	129 (40.5%)	293 (30.4%)
Right medial jointline tender	137 (27.7%)	562 (21.0%)	150 (41.6%)	298 (30.9%)
Right knee crepitus	185 (37.5%)	916 (34.3%)	219 (61.0%)	510 (52.9%)
Right knee patellar grind	79 (16.2%)	218 (8.7%)	110 (31.0%)	193 (20.0%)
Left patellar tendon pain	102 (20.8%)	296 (11.0%)	114 (30.9%)	174 (18.0%)
Left knee flexion pain	96 (19.5%)	289 (10.8%)	149 (40.5%)	293 (30.4%)
Left lateral jointline tender	116 (23.6%)	395 (14.7%)	126 (34.2%)	233 (24.1%)
Left medial jointline tender	125 (25.4%)	591 (22.0%)	152 (41.3%)	336 (34.7%)
Left knee crepitus	185 (37.6%)	912 (34.0%)	223 (61.1%)	516 (53.4%)
Left knee patellar grind	83 (17.1%)	233 (8.7%)	116 (32.2%)	210 (21.8%)

control at all time points ($p < .001$). When given individually, these biologic agents had less pronounced effect on the preservation of Safranin O staining, yet remained better than other tested agents.

Conclusions: PTOA therapies will be most effective if a multi-mechanistic approach is utilized to prevent chondrocyte death and matrix loss and to stimulate reparative responses. Combination of treatments that target three distinct mechanisms: cell membrane protection (P-188), inhibition of catabolism (anti-TNF- α), and stimulation of anabolic responses (OP-1) appears to be the most promising approach to biologic intervention of PTOA. Importantly, this Triad shows promise for rapid translation into clinical settings since all three factors have already been approved by the FDA for other use.

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INTRA-ARTICULAR BLOOD COAGULATION AGGRAVATES JOINT DAMAGE AFTER A BLEED IN A CANINE *IN VIVO* MODEL

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Purpose: Joint bleeding due to trauma, major joint surgery, or hemophilia leads to joint damage. However, it is unclear if there are differences between coagulating blood and anticoagulated blood with respect to joint degeneration, especially *in vivo*. Therefore, we evaluated in a canine *in vivo* model whether intra-articular blood exposure is more destructive in case of coagulating blood compared to anticoagulated blood, and whether inflammation plays a role in the cartilage damaging process.

Methods: In 7 dogs left knees were injected with coagulating blood 4 times a week in week 1 and 4; right knees with saline. In 7 other dogs anticoagulated, heparinized blood was injected with heparinized saline as control. Ten weeks after the last injection cartilage matrix turnover and synovial inflammation were analyzed *ex vivo*. To study cartilage damage without involvement of synovial inflammation, healthy full thickness human cartilage explants were exposed *in vitro* to coagulating and anticoagulated blood for 4 days ($n=6$). Cartilage matrix turnover was determined at day 16.

Results: Canine knees injected with coagulating blood showed an increase of both newly formed and total (resident) glycosaminoglycans of 9% and 15%, respectively ($p=0.04$ and $p=0.01$, respectively), as well as a decreased proteoglycan content of 6%. Injection with coagulating and anticoagulated blood caused an increase of proteoglycan synthesis rate of 24% and 14%, respectively ($p=0.01$ and $p=0.04$, respectively), as a characteristic of (ineffective) repair activity. Intra-articular injections with coagulating blood led to more synovial inflammation on macroscopy as well as histology ($p<0.001$ and $p=0.01$, respectively), in contrast to knees injected with anticoagulated blood. Coagulation of blood *in vitro* resulted in more cartilage damage compared to anticoagulated blood. This was expressed as more reduction of proteoglycan synthesis rate by coagulating blood compared to anticoagulated blood (17% difference; $p=0.05$), and a higher release of proteoglycans after exposure to coagulating blood compared to anticoagulated blood (94% difference; $p=0.01$).

Conclusions: This study shows that coagulating blood causes more long-lasting *in vivo* joint damage than anticoagulated blood; directly on cartilage and via inflammation. In case of joint surgery prolonged anticoagulation might limit the harmful effects of intra-articular blood. Moreover, aspiration of blood would prevent joint damage.

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DEPLETION OF PERLECAN DOMAIN I HEPARAN SULFATE REGULATES FIBROBLAST GROWTH FACTOR ACTIVITY IN CARTILAGE AND PROTECTS AGAINST CARTILAGE LOSS AND OSTEOPHYTE DEVELOPMENT IN POST-TRAUMATIC OSTEOARTHRITIS

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Purpose: Fibroblast growth factors (FGF) -2 and -18 have been suggested as potential therapeutic agents in osteoarthritis (OA). FGF-18 promotes chondrogenesis and cartilage repair *in vitro* and in animal models. FGF-2 has demonstrated both chondroprotective and pro-degradatory properties, potentially dependent upon variable receptor expression. Both FGF-2 and -18 signalling require heparan sulfate (HS) as a coreceptor. Given its pericellular localisation, perlecan (HSPG2) has been implicated as a critical agent in FGF-2/18 activities. We directly investigated the role of perlecan domain I HS in the onset and progression of post-traumatic OA, and whether the effects can be attributed to altered FGF-2/18 signaling in articular cartilage.

Methods: Male 10-weeks old wildtype (WT) and Hspg2 exon-3 null (in which the domain I HS chains are ablated) mice underwent unilateral destabilisation of the medial meniscus (DMM). Cartilage damage, subchondral bone sclerosis and osteophyte development were scored 4- and 8-weeks post-operatively. Femoral head cartilage (3 week old mice both genotypes) were cultured \pm IL-1 (10ng/ml) \pm FGF-2 or -18 (0, 1, 10, 100ng/ml) for 4 days. Sulfated glycosaminoglycan (sGAG) released into the media was quantified. Cartilage explant gene expression was analysed by real time PCR.

Results: There were no differences in cartilage aggrecan loss or structural damage 4 weeks post-DMM surgery between genotypes. At 8 weeks exon-3 null mice had significantly less cartilage erosion than WT (Fig 1A). The increase in subchondral bone sclerosis post DMM did not differ between genotypes, but osteophyte size was significantly reduced in exon-3 null mice at 8 weeks (Fig 1B).

There were no genotypic differences in either basal- or IL-1-stimulated sGAG release from cartilage explants. FGF-2 did not alter sGAG release \pm IL-1 in either genotype at any dose. FGF-18 alone did not alter sGAG release in either genotype, but significantly attenuated IL-1-induced sGAG loss ($\sim 35\%$) only in exon-3 null cartilage.

There were no differences between genotype in gene expression in control or IL-1 cultures. FGF-2 alone was generally "pro-catabolic", up-regulating Adamts4 & 5 and Mmp13 mRNA in exon-3 null cartilage. In the presence of IL-1, FGF-2 decreased WT RNA yield and Gapdh mRNA, suggestive of the detrimental effect of FGF-2 in injured cartilage. FGF-18 in the absence of IL-1 decreased Adamts4 in both genotypes, and markedly suppressed Mmp2 expression only in exon-3 null cartilage. Unlike FGF-2, FGF-18 did not affect cell viability in either genotype in the presence of IL-1.

Figure 1. Cartilage erosion (A) and osteophyte size (B) in surgically induced OA in WT and Hspg2 exon-3 null mice ($n = 17-20$ /group).

Conclusion: Increased mRNA expression in response to FGF-2 (Adamts4/5, Mmp13) and FGF-18 (Mmp2) in exon-3 null cartilage confirmed the importance of perlecan domain I HS, suggesting it acts by sequestration and reduced presentation to receptors in cartilage. The *in vitro* data suggests that reduced cartilage erosion in exon-3 null mice post DMM surgery may be associated with increased FGF-18 rather than altered FGF-2 signalling in chondrocytes in the absence of perlecan domain I HS. The mechanism behind reduced osteophyte size in exon-3 null mice is unclear but may result from altered stem cell differentiation, endochondral ossification, and/or activities of growth factors (e.g. TGF β) implicated in osteophyte development. We suggest that the lack of perlecan domain I HS chains negates FGF-18 sequestration, altering signalling effects and *in vivo* cartilage remodeling. Controlling the synthesis and/or turnover of chondrocyte perlecan and/or HS substitution may provide a therapeutic target alone or in combination with FGF-18 in the treatment in OA.

A. Cartilage Erosion B. Osteophyte Size

